Human limbal mesenchymal cells support the growth of human corneal epithelial stem/progenitor cells.

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Public Summary:
PURPOSE: We tested the viability of human limbal mesenchymal cells (LMCs) to support the expansion of human corneal epithelial stem/progenitor cells (LSCs). METHODS: Human LMCs were isolated from sclerocorneal tissue using collagenase A. Primary limbal epithelial cells (LECs) in the form of single cell suspension or cell clusters were cocultured on a monolayer of either 3T3 cells (control) or LMCs (SC-LMC culture). The LEC clusters also were grown directly on LMCs (CC-LMC culture) and in an optimized 3-dimensional culture method (3D CC-LMC culture). Colony-forming efficiency (CFE) and LEC proliferation were analyzed. The phenotype of the cultured LECs was assessed by their expression level of putative stem cell markers and a differentiation marker by qRT-PCR and immunocytochemistry. RESULTS: The LECs in the SC-LMC culture had a very limited growth and the stem/progenitor phenotype was lost compared to the control. Growth and cell morphology improved using the CC-LMC culture. The 3D CC-LMC culture method was the best to support the growth of the LSC population. Expression of ATP-binding cassette family G2 and DeltaNp63 at the mRNA level was maintained or increased in CC-LMCs and 3D CC-LMC cultures compared to the control. The percentage of the K14(+) and K12(+) cells was comparable in these three cultures. There was no significant difference in the percentage of p63alpha high expressing cells in the control (21%) and 3D CC-LMC culture (17%, $P > 0.05$). CONCLUSIONS: Human LMCs can substitute 3T3 cells in the expansion of LSCs using the 3-dimensional culture system.

Scientific Abstract:
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